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THE ACTION OF SEROTONIN IN THE RAT HIPPOCAMPAL SLICE PREPARATION

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SUMMARY

1. Intracellular activity was recorded from neurones in the CA1 pyramidal layer of slices of rat hippocampus maintained *in vitro*.

2. Application of 5-HT in a droplet or via ionophoresis produced a 3–5 mV hyperpolarization associated with a 30 % decrease in input resistance.

3. The response to 5-HT was minimal with a drop concentration of 1 μM and maximal with 100 μM. The responses appeared to be blocked by methysergide applied in the superfusion medium.

4. The responses to 5-HT were minimal when the drug was applied in the apical dendritic region and maximal when it was applied near the soma.

5. 5-HT produced no substantial changes in e.p.s.p.s evoked by stimulation of the Schaffer collateral–commissural system or in i.p.s.p.s which were occasionally encountered following stimuli to the stratum radiatum.

6. The responses to 5-HT are true post-synaptic responses and are not indirect effects since they are present in a Ca^{2+}-deficient Mg^{2+}-enriched medium which blocks synaptic transmission.

7. The responses to 5-HT were not dependent on extracellular Cl^- concentration.

8. These experiments indicate that 5-HT produces its effects in the rat hippocampus by activating K^+ channels.

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is one of the most extensively studied neurotransmitters in the mammalian brain. It is contained in the raphe nuclei of the brain stem which project to the rest of the brain and spinal cord. With the exception of a few studies (Barasi & Roberts, 1974; Bradley & Briggs, 1974; Couch, 1976; Roberts & Straughan, 1967), the effects of ionophoretically administered 5-HT on neurones in the mammalian central nervous system are reported to be mainly depressant (Bloom, Hoffer, Siggins, Barker & Nicoll, 1972; Phillis, Tebēcis & York, 1967; Roberts & Straughan, 1967; Sastry & Phillis, 1977; Wang & Aghajanian, 1977; Weight & Salmoiraghi, 1968). Little is known about the mechanism of action of 5-HT in the mammalian brain. This contrasts with the action of 5-HT in invertebrate ganglia where several different ionic mechanisms have been characterized (Gerschenfeld & Paupardin-Tritsch, 1974; Pellmar & Carpenter, 1979; Sawada & Coggeshall, 1976; Cottrell & Macon, 1974; Walker & Woodruff, 1972).

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The hippocampus of the rat is among the richest in 5-HT receptors in the brain (Bennett & Snyder, 1976). It contains 5-HT (Gage, Thompson & Valdes, 1978) and receives a monosynaptic innervation from the median raphe nucleus (Azmitia & Segal, 1978; Conrad, Leonard & Pfaff, 1974; Moore & Halaris, 1975; Segal & Landis, 1974). Stimulation of the raphe or ionophoresis of serotonin inhibit spontaneous hippocampal activity (Segal, 1975, 1976), effects which are partially blocked by several serotonin antagonists, e.g. methysergide. The hipiocampal slice preparation, first described by Yamamoto (1972), offers the advantages of studying an organized structure under *in vitro* conditions (Andersen, Silfvenius, Sundberg, Sveen & Wigstrom, 1978; Schwartzkroin, 1975). I have used the rat hippocampal slice preparation to study effects of serotonin on membrane properties of neurones in the pyramidal layer of region CA1 and wish to report that serotonin appears to activate K^+ channels in these neurones.

METHODS

Adult (250–350 g) male Wistar rats were decapitated and their brains rapidly removed and placed in Krebs solution. The right hippocampus was dissected out and placed on wet filter paper. Slices of 350 μm were cut with a McIlwain tissue chopper at an angle of roughly 30° normal to the longitudinal axis of the hippocampus and transferred into a Petri dish containing Krebs solution. The procedure was completed within 3–4 min. Slices were then transferred to the recording chamber (Andersen, Silfvenius, Sundberg, Sveen & Wigstrom, 1978; Schwartzkroin, 1975) and placed on lens paper covering a nylon net where they were continuously superfused with oxygenated (95% O_2, 5% CO_2) Krebs solution. The fluid level was adjusted to the level of the slices. In addition, the slices were superfused from above with humidified gas mixture (flow rate of 0·2 l./min). Normal Krebs solution contained (mM) NaCl 124, KCl 5, KH_2PO_4 1·25, $NaHCO_3$ 26, $MgSO_4$ 2, $CaCl_2$ 2 and D-glucose 10. The solution had pH 7·4 and was equilibrated with a gas mixture of 95% O_2, 5% CO_2 before use. In some experiments Ca^{2+} concentration in the superfusion medium was 0·2 mM and Mg^{2+} 4 mM. To obtain a low Cl^- solution, NaCl was replaced by 124 mM-Na propionate. The temperature in the recording chamber was maintained at 32–33 °C with a regulated heating element.

Intracellular recording was made with glass micropipettes filled with 4 M-K acetate and having a d.c. resistance of 70–150 MΩ. Signals were fed through a M-707 WPI microprobe system, displayed on an oscilloscope and continuously plotted on a Brush DC chart recorder. Input resistance was measured by passage of 0·5 Hz 100 msec 0·5 nA hyperpolarizing pulses through the recording electrode. The bridge balance was routinely checked and adjusted when necessary. Responses to the hyperpolarizing pulses were averaged with an Ortec signal averager and plotted (Fig. 1).

A monopolar tungsten micro-electrode was used to stimulate excitatory afferent pathways. The electrode was routinely placed in stratum radiatum, 2–3 mm away from the recorded cell (Fig. 1). Occasionally, a stimulating electrode was placed in the stratum oriens or alveus. Current pulses (0·2 msec, 0·5–1 Hz, 10–100 μA) were applied by a Devices stimulator.

Drugs were applied (*a*) in a droplet, (*b*) via inophoresis, (*c*) in the superfusion medium. 5-HT was routinely applied in a drop; it was dissolved in Krebs solution prior to use. A coarse pipette connected to a Leitz manipulator and a microdrive were used to apply droplets of 1–20 nl. to the surface of the slice. Drop size was estimated relative to the width of the pyramidal layer. In these experiments, no more than one cell was usually recorded in a slice. For ionophoresis, three of the four outer barrels of a five-barrel micropipette (tip diameter 2–5 μm) were filled with 0·2 M-5-hydroxytryptamine creatinine sulphate (5-HT), 1 M-L-noradrenaline-hydrochloride (NA) and 1 M-γ-aminobutyric acid (GABA). The drugs were dissolved in distilled water and the pH adjusted to 5–5·5. The fourth barrel was filled with a 3 M-NaCl and served for continuous current neutralization (Weight & Salmoiraghi, 1968). In experiments where drugs were applied by superfusion, the fluid flow rate was monitored and set at 0·2 ml./min. As determined experimentally (with light absorbance of coloured water measured with a spectrometer), 50% of the

fluid in the experimental chamber (2 ml.) was replaced in 8–10 min and the solution was completely replaced in 18–20 min. All electrodes were placed under visual control using a Nikon dissecting microscope.

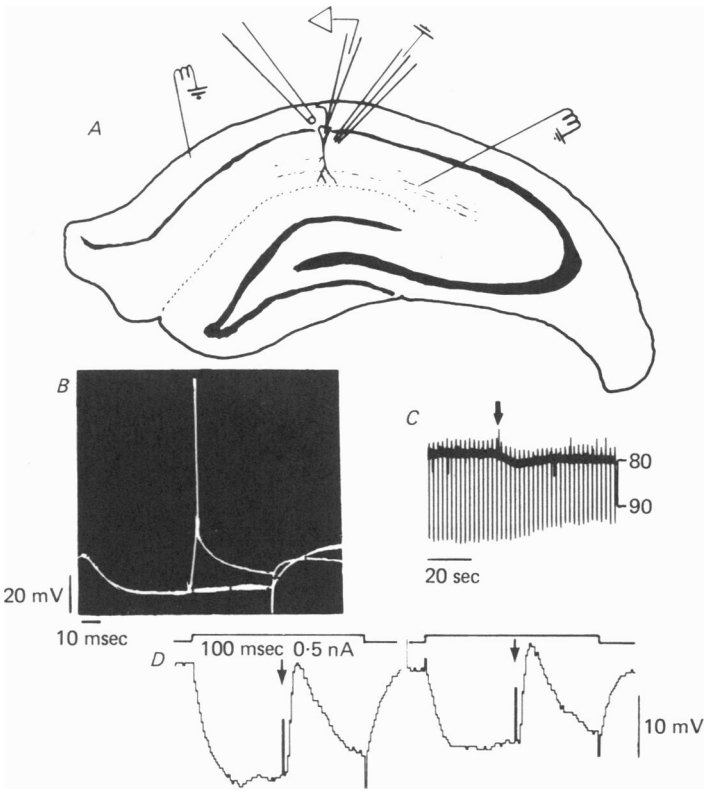

Fig. 1. *A*, the arrangement of stimulating and recording electrodes in the slice preparation. Intracellular recording was made from cells in the pyramidal layer of region CA1. A monopolar stimulating electrode was routinely placed in the stratum radiatum (right) to activate the Schaffer collateral–commissural system. Another stimulating electrode (left) was sometimes placed in the alveus, to invade antidromically the recorded cells. A five-barrel micropipette or a single wide-bored pipette were placed at various distances from the recording electrode. *B*, a typical oscillographic record of a response of a cell to a hyperpolarizing current pulse and an action potential arising from an e.p.s.p. produced by stimulation of the stratum radiatum. *C*, a continuous record of membrane potential and responses to hyperpolarizing current pulses applied at a rate of 0·5 Hz before and after a 0·5 mM drop application of 5-HT (arrow). *D*, averaged responses to current pulses and stimulation of the stratum radiatum (arrow) before (left) and after (right) 5-HT application. Same cell as in *C*. In this and following Figures, four successive pulses were averaged with a 1 msec bin width.

RESULTS

Stable intracellular recordings were made from seventy-nine neurones. Membrane potential was usually unstable for the first few minutes after impalement at which time steady hyperpolarizing currents (up to 1 nA) were used to improve recordability. These currents were removed thereafter and stable recordings could then be maintained for up to 4 h. Resting membrane potential was measured upon withdrawal

of the recording electrode and amounted to $-65{\cdot}5 \pm 1{\cdot}67$ mV (mean ± S.E. of mean) (range 45–85 mV). Input resistance was measured routinely with a 0·5 nA hyperpolarizing current pulse and amounted to $30{\cdot}0 \pm 1{\cdot}24$ MΩ (range 12–60 MΩ). The time constant of the voltage deflexion was $22{\cdot}1 \pm 3$ msec.

Since membrane potential could not be determined reliably until after termination of the recording session, the viability of the recorded cell was determined by the magnitude of its input resistance and by its ability to respond to orthodromic stimulation and depolarizing pulses. Orthodromic stimulation evoked e.p.s.p.s which were graded with stimulus intensity and sometimes reached peak amplitudes of 25 mV. If the e.p.s.p.s were sufficiently large, impulses were discharged. The mean amplitude of such spikes was $82{\cdot}1 \pm 2{\cdot}1$ mV (range 55–105 mV). Spontaneous activity was not encountered in most cells in normal conditions but when present, there was a wide range of firing rates (0·1–10 Hz).

Occasionally, a drop of 70–80 mV in potential was encountered upon advancement of the electrode in the tissue. This was not accompanied by a noticeable resistance change or by any reversal in the polarity of the population spike normally detectable in extracellular recordings (Andersen *et al.* 1978). Such potential changes were therefore attributed to penetration of glial cells and were ignored.

Effects of 5-HT

5-HT was routinely applied in a drop concentration of 5×10^{-4} M. In thirty-two of thirty-seven cells, 5-HT produced a hyperpolarization accompanied by a reduction in input resistance. The magnitude of the hyperpolarizing response was variable and there appeared to be a significant negative correlation between the resting potential and the hyperpolarizing response ($r = -0{\cdot}75$). The response ranged between 0 and -9 mV (mean $3{\cdot}76 \pm 0{\cdot}69$ mV). As opposed to the potential changes, the resistance changes were more consistent. There was a $31 \pm 3\%$ drop in input resistance. The resistance change was not a mere reflexion of hyperpolarization at a given potential since hyperpolarization of the membrane with current to the same potential did not mimic the effects of 5-HT. The reduction in resistance was accompanied by a fall in membrane time constant to $13{\cdot}2 \pm 1{\cdot}9$ msec.

The time course of the effects of 5-HT was fairly slow. The hyperpolarization started within 1–2 sec after the drop application and reached a peak within $11{\cdot}5 \pm 1{\cdot}8$ sec, but there was a wide range of latencies to reach the peak (2–40 sec). Recovery was also variable and a return to base-line potential and resistance was seen within 2–20 min. Application of a second drop of 5-HT while the effects of the first one were still evident did not cause a further change in potential or resistance, indicating that the receptors for 5-HT were saturated.

A small depolarization occasionally followed the fall of the drop on the tissue (Fig. 2). In most cases, this lasted for 2–4 sec, after which a recovery of both potential and resistance was seen. In more severe cases the mechanical vibration caused a loss of the recorded cell.

Since input resistance appeared to be a more reliable indicator of the effects of 5-HT it was routinely used in the following experiments.

The effects of various drop concentrations of 5-HT on the membrane potential and resistance were measured in five cells. A drop concentration of 1 μM did not produce

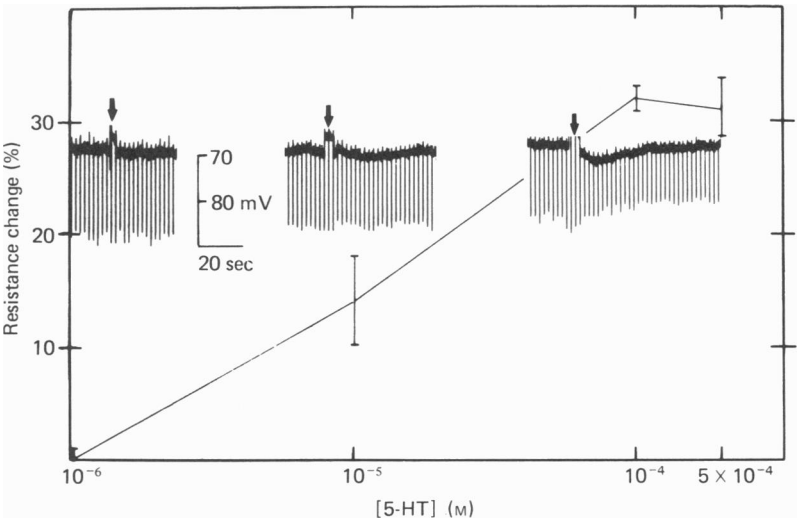

Fig. 2. A dose–response curve of the magnitude of change produced by a drop application of 5-HT. Inserts are three successive applications of the various concentrations on a single neurone, arrow = drop application. The scale is the same for all three tests.

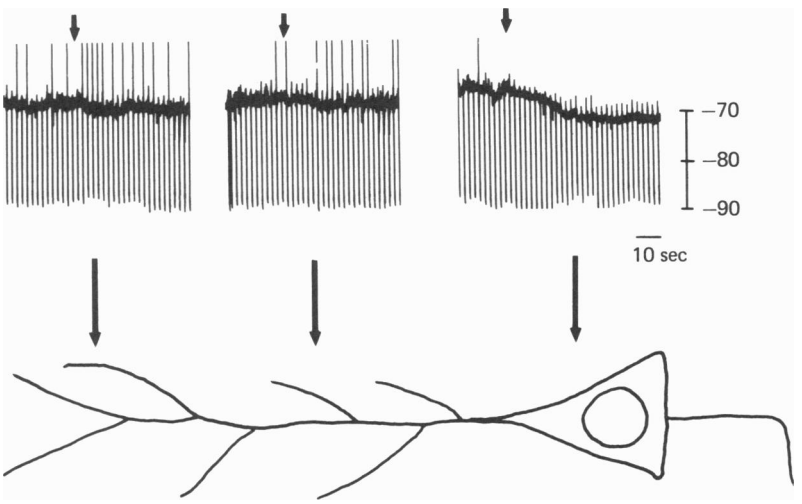

Fig. 3. The effects of application of 5-HT in three areas along the apical dendrites of a pyramidal neurone. 5-HT produced an effect only when applied on the soma. (Arrow: drop application).

any noticeable change in either potential or resistance (Fig. 2). 5-HT at 10 μM concentration reduced membrane resistance by 14 ± 4 %. Maximal effects on resistance were seen with 100 μM-5-HT (32 ± 1 %). At this concentration there was also a noticeable (3–4 mV) hyperpolarization.

The site of action of 5-HT was determined by applying small (1–8 nl.) droplets of 5-HT along the recorded neurone. It produced no effects when applied in the dentate molecular layer, just underneath the recorded cell, or in the stratum moleculare or

radiatum (Fig. 3). 5-HT applied on the stratum pyramidale and the adjacent oriens produced the maximal change in potential and input resistance observed previously (three cells).

The specificity of the effects of 5-HT on membrane potential and input resistance was tested by comparing 5-HT with GABA and noradrenaline, the two other putative

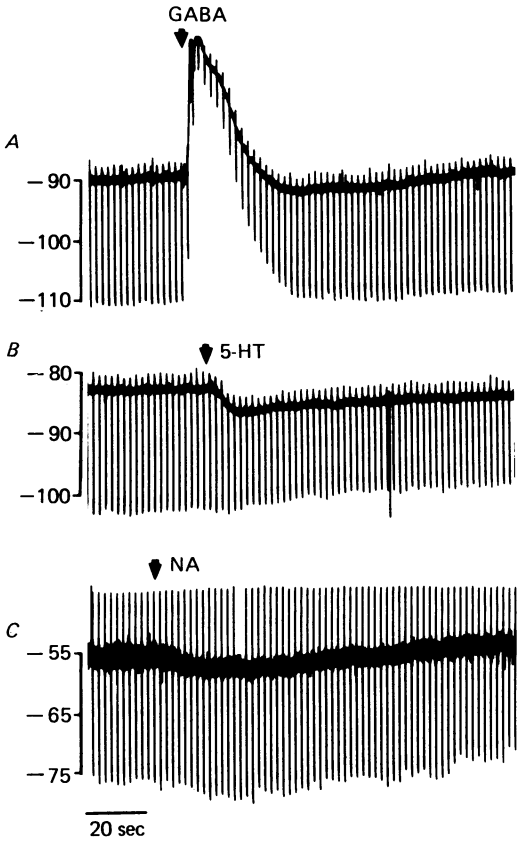

Fig. 4. The effects of 5-HT, GABA and NA on hippocampal neurones. *A* and *B* are two consecutive applications of GABA and 5-HT on the same neurone and *C* is from a different neurone. All three drugs were dissolved in Krebs solution prior to use to form a concentration of 0·1 mM. 5-HT application (arrow) caused the typical hyperpolarization associated with a moderate (30%) reduction in input resistance. GABA, in this cell, caused a 78% reduction in input resistance associated with a 20 mV depolarization. NA (arrow, *C*) produced a moderate hyperpolarization which was not associated with a conductance change.

inhibitory neurotransmitters in the rat hippocampus. The effects of GABA (four cells) and NA (five cells) were strikingly different from those of 5-HT (Fig. 4). Application of GABA (0·1 mM drop concentration) produced a fast (1–2 sec) 60–70% reduction in input resistance and a potential change which was dependent upon the resting membrane potential; with a more negative resting potential (i.e. −90 mV, Fig. 4), the cell was depolarized by GABA. The recovery from the effects of GABA

was also rather fast and could be seen within 10–20 sec. NA (0·1 mM) on the other hand, hyperpolarized the cell regardless of the resting membrane potential. The hyperpolarizing response was small (2–3 mV), had a slow onset (10–20 sec) and was associated with minor changes in input resistance (Fig. 4*C*). It therefore appears that though the three neurotransmitters inhibit spontaneous activity of hippocampal neurones, they may be doing it via three different ionic mechanisms.

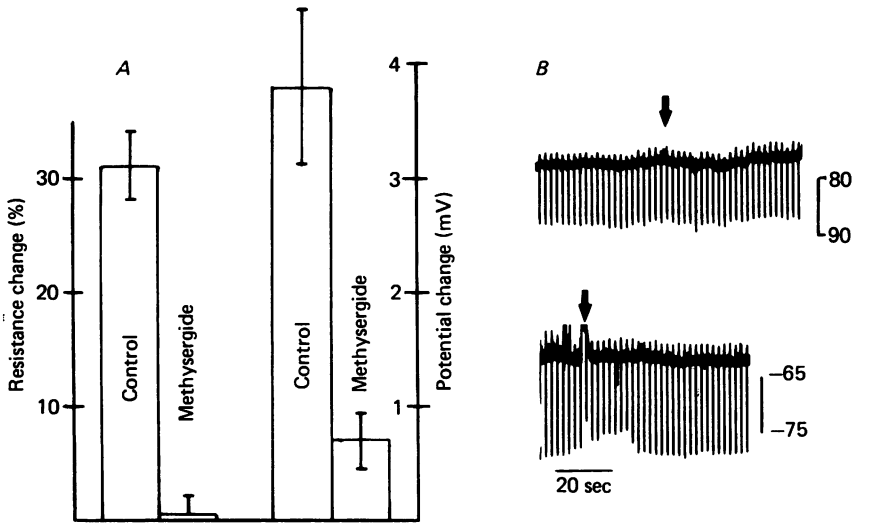

Fig. 5. An antagonism by methysergide (100 μM) of the effects of 5-HT. *A*, averages of resistance (left) and potential (right) changes produced in control and seven methysergide superfused slices. *B*, two examples showing the lack of effect of 5-HT in the presence of methysergide.

Antagonist effects

To test the assumption that 5-HT exerts its action via a 5-HT receptor, its antagonist methysergide (Segal, 1976; Nelson, Herbert, Bourgoin, Glowinski & Hamon, 1978) was used. Methysergide (0·1 mM) was added to the superfusion medium and tested in seven cells (Fig. 5). Methysergide had no systematic effect on membrane potential and there appeared to be a small (about 10 %) reduction in input resistance upon superfusion with the drug. 5-HT applied in a drop in the presence of methysergide caused no noticeable change in input resistance and only a small (less than 1 mV) hyperpolarizing effect on the membrane potential (Fig. 5). Responses to 5-HT returned to normal after substitution of the methysergide-containing medium with the normal medium.

Low Ca^{2+} experiments

The involvement of Ca^{2+} in the potential and resistance changes produced by 5-HT was tested in three cells. Extracellular recordings of population spikes before and after superfusion with low Ca^{2+}, high Mg^{2+} solutions indicated that synaptic transmission ceased within 20 min after onset of the low Ca^{2+} superfusion. The cells were slightly depolarized and emitted spontaneous action potentials (Fig. 6). Under

these conditions, 5-HT produced the same or even larger effects on membrane potential and input resistance ($38 \pm 4\%$) as compared with the effects observed in the presence of normal medium. It appears therefore that 5-HT exerts its effects postsynaptically by a Ca^{2+}-independent process.

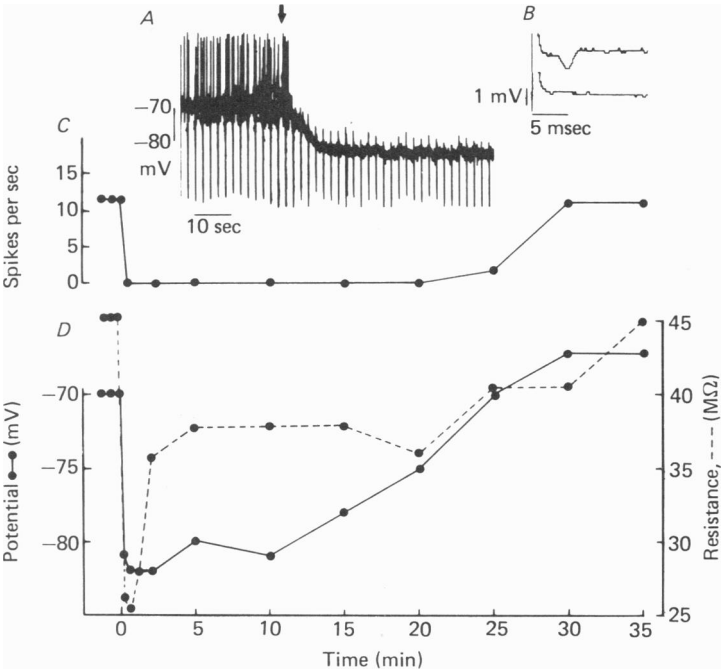

Fig. 6. The action of 5-HT in Ca^{2+}-deficient Mg^{2+}-enriched medium. *A*, example of the effects of 5-HT (at arrow) in a spontaneously active neurone. *B*, extracellularly evoked population spike recorded at pyramidal cell layer before (top trace) and after (bottom trace) the change from normal to a Ca^{2+}-deficient medium. *C*, changes in spontaneous action potentials induced by 5-HT added at time zero. *D*, changes in potential and input resistance over time. Abscissa (time) is the same for *C* and *D*. *A*, *C*, and *D* are from the same cell, and *B* is from the same slice.

Ionophoresis experiments

Ionophoresis experiments were performed to ensure that the observed effects of 5-HT were not a product of the mode of its administration. 5-HT was administered by ionophoresis onto eighteen cells. Altogether this method proved to be less effective than the drop technique, and produced a change in only thirteen of eighteen cells. The over-all change in membrane potential was $-2{\cdot}4 \pm 0{\cdot}5$ mV and in resistance $13 \pm 2\%$. While these changes are similar to those produced by the 5-HT in a drop, they are none the less smaller. Also, a prolonged application was needed to produce these effects (Fig. 7). Furthermore, care had to be taken to avoid artifacts due to current alone (Fig. 7*D*). Such artifacts were easily identified, were usually seen with larger ejection currents and had the same magnitude when the recording electrode was just outside a cell.

The relatively small effect produced by ionophoretically administered 5-HT was

not unexpected in view of the fact that the ionophoresis and recording electrodes were driven independently 100–300 μm apart and that the drug was applied only in a limited area, not always where its effects were maximal. In any event, there were no qualitative differences between the two modes of application of 5-HT.

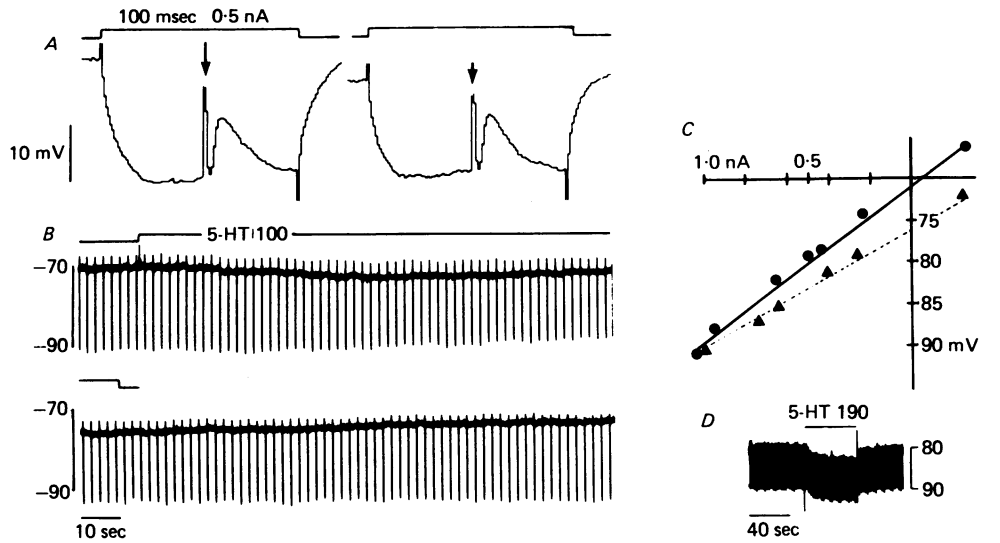

Fig. 7. The effects of ionophoretic application of 5-HT on a hippocampal neurone. *A*, computer averages of responses to an hyperpolarizing current pulse and orthodromic stimulation (arrow) before, left and during, right, the injection of 5-HT. Note the differences between the histograms in resting potential, response to the hyperpolarizing pulse but no difference between the e.p.s.p.s. *B*, a continuous record of the membrane potential and current responses during 5-HT ionophoresis with current of 100 nA. *C*, a current–voltage curve of a cell before (dots) and during (triangles) ionophoresis of 5-HT. *D*, an example of a possible artifact produced by current ionophoresis. Note the sharp changes in the potential at the onset and offset of the 5-HT ejection current.

Effects of 5-HT on e.p.s.p.

Large e.p.s.p.s were produced by stimulation of the excitatory Schaffer collateral–commissural fibres which terminate in the stratum radiatum (Andersen, Bliss & Skrede, 1971; Andersen *et al.* 1978). Increasing the stimulation current resulted in the generation of action potentials, as evidenced by the appearance of extracellularly recorded population spikes (Andersen *et al.* 1971). The effects of 5-HT on these e.p.s.p.s were tested systematically in fourteen cells. In seven of these there was no change (less than 10 %) in magnitude of the averaged e.p.s.p. (Fig. 8*C*) after application of 5-HT. In two cells there was a moderate (20 %) reduction, and in four cells there was a significant (40–50 %) reduction in magnitude of e.p.s.p. In one cell there was a 40 % increase in e.p.s.p. In all cells there was no apparent correlation between the effects of 5-HT on potential, resistance and e.p.s.p. When measured extracellularly, the magnitude of the population spikes was reduced by 5-HT. This effect was more pronounced with low stimulation currents and became smaller with higher currents (Fig. 8*D*).

Effects of 5-HT on i.p.s.p.

I.p.s.p.s were occasionally produced by stimulation of the stratum radiatum or oriens. The reversal potential of these i.p.s.p.s lay between −60 and −65 mV; thus, when cells had more negative resting potentials, i.p.s.p.s were not easily distinguishable from e.p.s.p.s. Applications of 5-HT usually hyperpolarized the cell to a level

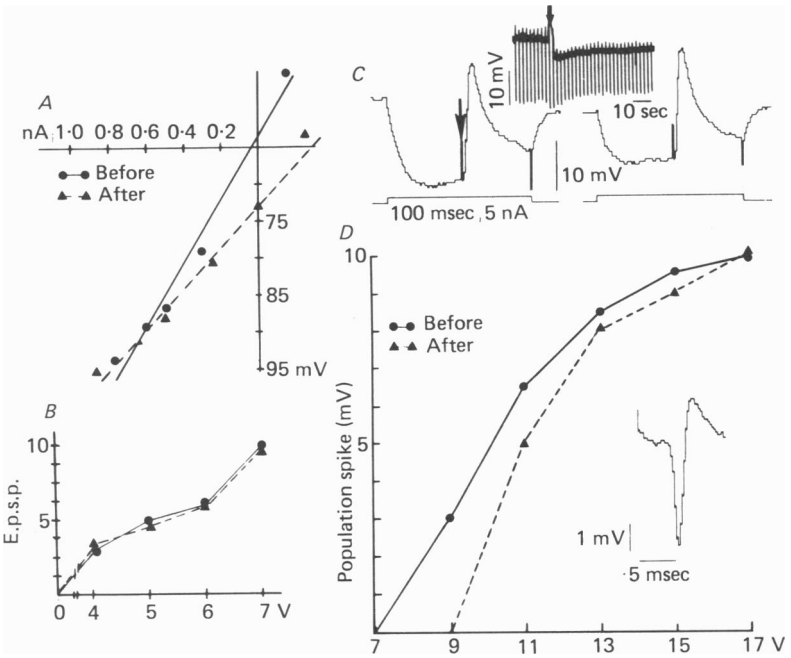

Fig. 8. The effects of 5-HT on e.p.s.p.s. *A* and *B* tests made in the same cell. *A*, current–voltage plot demonstrating a reduction in input resistance associated with a 8 mV hyperpolarization. Reversal potential is around −90 mV. *B*, a strength curve measuring the magnitudes of e.p.s.p.s produced by stimulation of the Schaffer collateral–commissural system before (dots) and after (triangles) drop application of 5-HT. *C*, a specimen record of the effect of 5-HT on membrane potential, resistance and e.p.s.p. *D*, the effects of 5-HT on population spikes recorded at the pyramidal cell layer. Insert demonstrates a population spike average.

more negative than the reversal potential of the i.p.s.p. The i.p.s.p.s could then be restored by applying a depolarizing current to the cell. This was observed in several cells and was studied systematically in one cell (Fig. 9). It thus appears that the 5-HT hyperpolarizations are not mediated by the same mechanism that mediates the i.p.s.p. in the hippocampus.

The ionic basis of 5-HT action

5-HT produced hyperpolarization associated with an increased conductance in most cells tested. The change in conductance of one of two possible ions, Cl^- or K^+, may mediate 5-HT action. The reversal potential for 5-HT action was −85–−90 mV

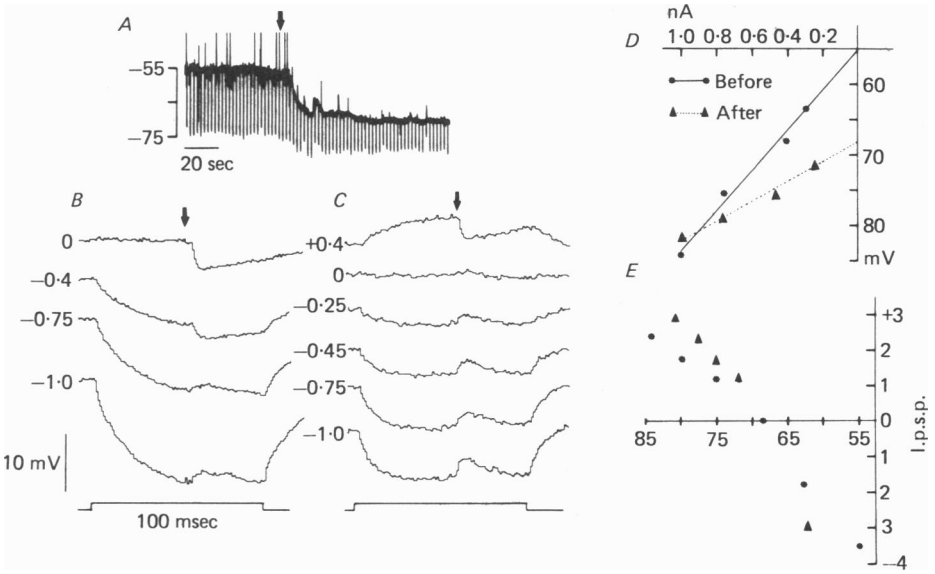

Fig. 9. Effects of 5-HT on i.p.s.p. *A*, a specimen record of the cellular responses to 5-HT (arrow). A marked hyperpolarization associated with a decrease in input resistance was recorded. *B*, i.p.s.p. was produced by stimulation near the pyramidal layer. Inhibitory stimuli were applied simultaneously with a series of hyperpolarizing current pulses (nA shown) to measure the reversal potential of the i.p.s.p. (−68 mV). *C*, same after 5-HT; in this case the membrane potential was slightly below the i.p.s.p. reversal potential and no i.p.s.p. was seen. An i.p.s.p. could still be produced by applying the stimulation on top of a depolarizing current pulse (+0·4 nA). *D*, a current–voltage plot of the cell's responses to a series of hyperpolarizing pulses, before (dots) and after (triangles) 5-HT application. The reversal potential for 5-HT is about 83 mV. *E*, magnitude of the i.p.s.p. at various potential levels before (dots) and after (triangles) 5-HT application, indicating a reversal potential for i.p.s.p. that is not changed by 5-HT.

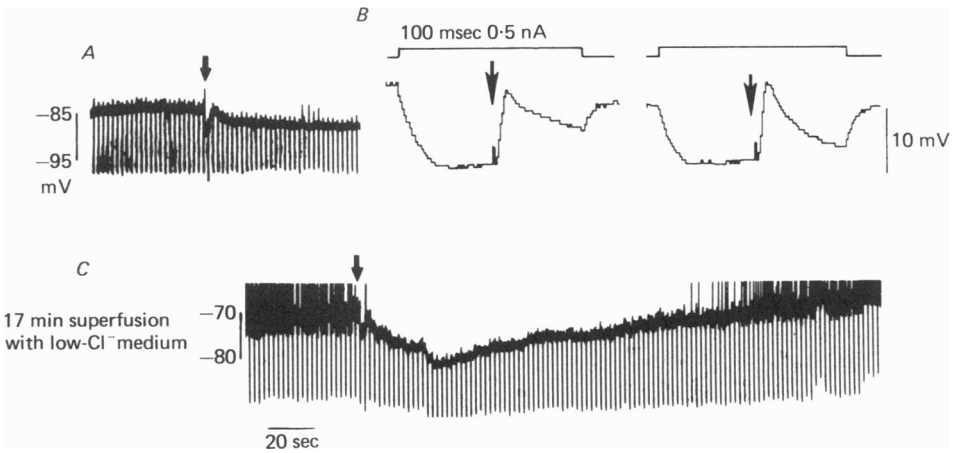

Fig. 10. The effects of a low-Cl^- medium on cellular responses to 5-HT. *A*, the cellular response to 5-HT (arrow) in a normal superfusion medium. *B*, averaged responses of the cell to the application of constant hyperpolarizing current pulses. *C*, the same cell, after replacing NaCl with Na propionate. (Note that only the bases of the spikes are shown.) Note the change in resting potential, the high spontaneous activity and the slight increase in input resistance, produced by the absence of Cl^- ions.

(Figs. 7*C*, 8*A* and 9*D*), which is more negative than that of the i.p.s.p. Since i.p.s.p.s in the hippocampus are probably produced by an increase in Cl^- conductance (Yamamoto, 1973) it is unlikely that Cl^- conductance is involved in 5-HT action. This possibility was further tested by lowering Cl^- concentration in the superfusion medium. Replacing NaCl with Na propionate depolarized the tested neurones by 5–15 mV (Fig. 10). This was associated with an increase in spontaneous activity, but

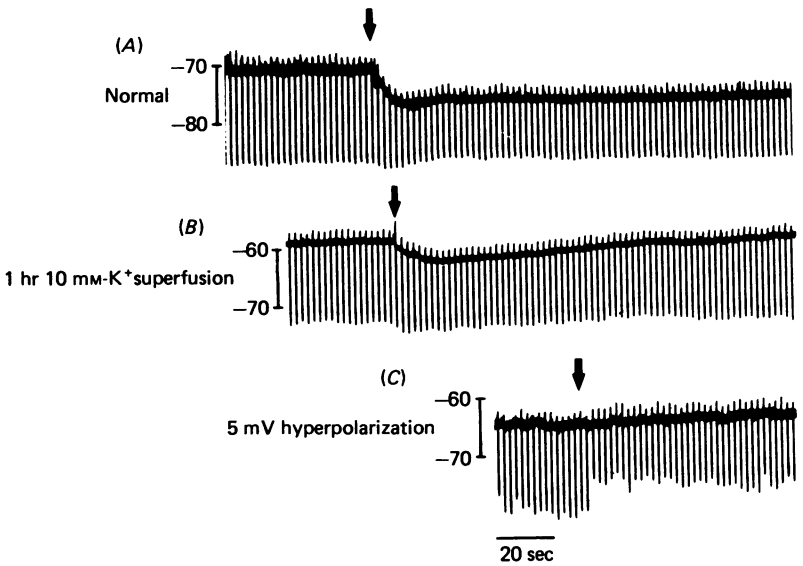

Fig. 11. The effect of extracellular K^+ on responses to 5-HT. *A*, the response to 5-HT in normal (5 mM) K^+ medium. *B*, same cell, after superfusion with 10 mM-K^+. Note the change in potential (+10 mV) and the smaller change in potential produced by 5-HT. *C*, same cell, superfused with 10 mM-K^+, hyperpolarized with continuous current by 5 mV. 5-HT no longer produced a hyperpolarization although a resistance change is evident.

with no clear changes in input resistance. The potential changes may suggest a role for Cl^- in resting membrane potential but may also result from loss of Cl^--dependent local inhibitory/hyperpolarizing input to the recorded neurone. 5-HT applied onto five cells maintained in low-Cl^- medium was as effective in hyperpolarizing the neurones as it was when normal medium was used. There was a $29 \pm 3\%$ reduction in input resistance and a $3{\cdot}8 \pm 1$ mV hyperpolarization. Interestingly, the duration of 5-HT action in low-Cl^- medium was shorter than in the normal case.

The lack of involvement of Cl^- channels in 5-HT action leaves K^+ channels as the main possible mediators of this effect. Indeed the reversal potential of 5-HT action is in the range of the equilibrium potential of K^+ (Katz, 1966) in other tissues tested.

Attempts to reverse 5-HT-induced hyperpolarization by applying hyperpolarizing currents were made in nine cells maintained in normal (5 mM) K^+-containing medium. These attempts were only partially successful. For technical reasons, only 5–10 mV hyperpolarization could be produced and under these conditions only a 25–50% reduction in 5-HT hyperpolarization were recorded.

An attempt was then made to shift the K^+ equilibrium potential by increasing extracellular K^+ to 10 mM (2 cells). This increase in extracellular K^+ caused a 10 mV depolarization associated with a small resistance change (Fig. 11). Under these conditions a smaller hyperpolarizing action of 5-HT was recorded and when the cells were further hyperpolarized by passage of current, the 5-HT-induced hyperpolarization

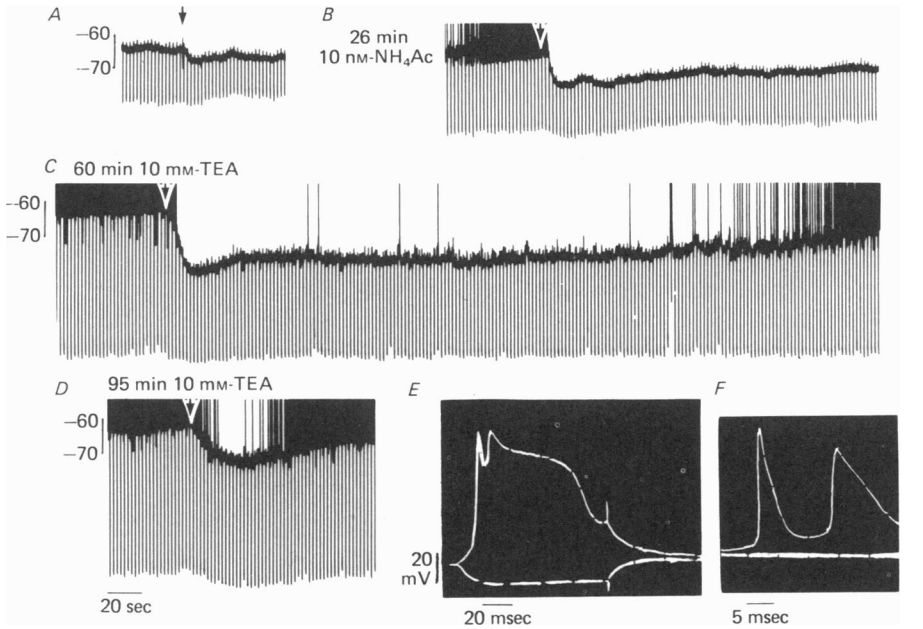

Fig. 12. The effects of TEA on cellular responses to 5-HT. All traces are from the same cell recorded over 3 hr. *A*, normal medium; 5-HT (arrow) produces a hyperpolarization associated with a decrease in resistance. *B*, after superfusion with 10 mM-ammonium acetate, which inactivates Cl^- channels, 5-HT still produces its effects on membrane potential and resistance. *C*, after superfusion with 10 mM-TEA. Note the large increase in resistance (to about 90 mΩ) and the high spontaneous activity. At this time spike shape was broadened to 5–10 msec (*F*) and a depolarizing pulse produced a large spike, lasting for nearly 100 msec (*E*). *D*, a presumably desensitized response to 5-HT after repeated application of the drug.

was no longer evident whereas resistance changes were still present. These experiments indicate that 5-HT may act by increasing conductance in K^+ channels. An attempt was then made to block K^+ conductance by tetraethylammonium (TEA) (six cells). Slices were superfused with a medium containing 10 mM-TEA (Fig. 12). Although applied extracellularly, TEA was effective in blocking Na^+-dependent K^+ channels. This was indicated by an increase (to 80–100 MΩ) in input resistance and by the large broadening of action potentials associated with a faster rise time of the spikes (Fig. 12*F*). The neurones exhibited high spontaneous firing rates, and when a depolarizing pulse was applied a broad spike lasting for 100 msec was generated (Fig. 12*E*).

Under these conditions, 5-HT still caused a marked hyperpolarization associated with a 25 ± 7 % decrease in resistance.

DISCUSSION

The present experiments have characterized cellular responses to 5-HT in the CA1 region of the rat hippocampus maintained *in vitro*. When applied ionophoretically or topically, 5-HT caused hyperpolarization associated with a marked decrease in input resistance. This effect had a rather slow time course, was maximal when the drop was applied in a concentration of 100 μM on the stratum pyramidale and not on the stratum radiatum and was not seen in the presence of a serotonin antagonist, methysergide.

The effects of 5-HT were not a function of the mode of its application since the topical or ionophoretic methods of administration produced qualitatively similar results. The ionophoretic technique could be expected to result in smaller effects as fewer receptors were probably activated by this method. Also, the distance between the recording and drug pipettes (100–300 μm) could cause long delays in the onset of 5-HT action. Attempts were made in preliminary experiments to apply 5-HT by superfusion. No clear effects could be seen, probably due to the slow and gradual arrival of the agonist molecules at the receptor sites, which may have evolved a process of desensitization. The possible desensitization was indicated in several experiments where repeated application of 5-HT at short intervals, before complete recovery, produced smaller and smaller physiological effects (Fig. 11).

There is an apparent dissociation between the localization of preterminal 5-HT-containing fibres of raphe origin in the hippocampus and the sites where 5-HT produced its maximal physiological effects. Previous anatomical studies (Azmitia & Segal, 1978; Conrad, Leonard & Pfaff, 1974; Moore & Halaris, 1975) have described 5-HT-containing fibres mainly in the distal end of the stratum radiatum and in the stratum lacunosum-moleculare of area CA1 of the hippocampus. Few, if any, fibres were found in the pyramidal layer. Drop application or, in some experiments, ionophoresis of 5-HT in the stratum moleculare or radiatum produced minor changes in membrane potential or input resistance of the tested neurones. It is possible that the light-microscopic localization of 5-HT in the hippocampus may not represent the locations of the serotonergic synapses. Indeed, such a disparity was described in a recent study on the monoaminergic innervation of the neocortex (Lidov, Molliver & Zecevic, 1978); monoaminergic fibres, visualized at the light-microscopic level, were abundant in the molecular layer (layer I) whereas synapses, visualized at the electron microscopic level, were localized mainly in layer IV, a cell-body layer. It is still possible that 5-HT exerts a specific action when released in the molecular layer as well but such an effect was not detected because of the distance between the recording site and the remote dendrites. It is also possible that the effects of 5-HT in the remote dendritic region are presynaptic. Such a possibility should be studied in further experiments.

There are three aspects to the possible specificity of 5-HT action.

(*a*) Is 5-HT activating a receptor for other inhibitory neurotransmitters, NA or GABA? This is unlikely since, although all three drugs produce similar extracellular effects, i.e. cessation of spontaneous activity, their effects on membrane potential and input resistance are completely different: NA causes a long latency hyperpolarization that is associated with minimal changes in input resistance; GABA produces a fast

and marked reduction in input resistance and its reversal potential is similar to that of i.p.s.p.s and Cl^- equilibrium potential.

(*b*) Is 5-HT acting indirectly, by activating some neurones afferent to the recorded neurone? This is unlikely since 5-HT effects are present in slices superfused with a low-Ca^{2+} high-Mg^{2+} medium which was shown to block synaptic activity in the slice.

(*c*) Are the effects of 5-HT exerted directly on some ionic channels or are they mediated by a serotonergic receptor? Methysergide, which inhibits the binding of [^{3}H]5-HT to synaptosomal membranes (Nelson *et al.* 1978) and antagonizes the extracellular action of 5-HT in several systems (Segal, 1976), was used. Although it had minimal effects by itself when applied by superfusion, or in preliminary experiments, in a droplet, it antagonized the effects of 5-HT. These experiments indicate that the effects of 5-HT are specific, and exerted on the recorded neurone and not elsewhere in the slice.

Several possible ionic mechanisms underlying the action of 5-HT were tested. It is unlikely that 5-HT activates Cl^- channels since the reversal potential for i.p.s.p.s which are probably Cl^--dependent (Davidson, 1976) is not the same as that for 5-HT. Also, replacement of Cl^- by propionate ions did not cause a reduction in 5-HT effects. The possibility that a Na^+–K^+ pump was activated by 5-HT is unlikely since the effects were present in two cells superfused with K^+-free medium (data not shown), a condition which interferes with the activation of the pump (Kobayashi & Libet, 1968). It is also unlikely that the K^+ currents activated by 5-HT are Na^+-dependent; TEA which affected the Na^+-dependent K^+ channels did not modify much the effects of 5-HT. Similarly, the K^+ channels do not appear to be Ca^{2+}-dependent because a Ca^{2+}-deficient medium causing loss of synaptic activity did not affect 5-HT actions.

5-HT may activate other mechanisms, in addition to its main effect. The observation that 5-HT did not change e.p.s.p.s in the cells tested, despite causing a large reduction in their input resistance, indicates that 5-HT may modulate responsiveness to excitatory afferents in a way which differs from its action on the soma. This possibility has to be explored in further experiments using intradentritic recordings in the hippocampus.

In summary, 5-HT appears to exert a specific and potent action towards rat CA1 neurones that is different from that of two other inhibitory neurotransmitters, GABA and noradrenaline. 5-HT appears to produce hyperpolarization and cessession of spontaneous activity by activating K^+ channels. These studies lend further support to the suggestion that 5-HT is an inhibitory neurotransmitter in the rat hippocampus.

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